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Original article

Hormonal responses associated with the nadir in blood glucose during graded cycling exercise

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Abstract

The response of glucoregulatory hormones associated with the nadir in serum glucose (i.e., glucose threshold) was examined during graded exercise to exhaustion in 22 physically active male participants. A comparison was also made between the glucose, lactate, and ventilatory thresholds. The mean (±SD) age, height, and body mass of the participants was 24.5 (±4.0) years, 182.0 (±9.1) cm, and 84.8 (±17.4) kg, respectively. Blood samples were collected at rest and during the final minute of each power output of a graded exercise test. The nadir in blood glucose concentration occurred at the same time as the lowest concentration of plasma glucagon, and both were significantly elevated at maximal exercise. Insulin response showed a significant decrease from rest but was maintained prior to the glucose nadir followed by a significant decrease. Cortisol was significantly elevated at maximal exercise intensity only. There was no difference in the power output and oxygen consumption (VO_2) at the glucose and lactate thresholds but both occurred at one power output prior to the ventilatory threshold. In conclusion, a nadir in serum glucose occurred in a predictable fashion in relation to other measures of anaerobic threshold. The nadir in blood glucose occurred prior to an increase in plasma glucagon concentration and a decrease in circulating insulin concentration during the re-establishment of euglycemia during graded exercise.

Keywords: Anaerobic threshold; Cortisol; Glucagon; Glucose threshold; Insulin

Introduction

The existence and measurement of the individual anaerobic threshold has been a controversial topic but remains a pivotal measure within exercise science[1–3] and has also been assessed in clinical populations, such as individuals with type 2 diabetes.[4] The measurement of blood lactate and ventilatory parameters during graded exercise are the most commonly used variables to determine anaerobic threshold and have been evaluated for reliability and validity.[3,5–8] The response of blood glucose to exercise has also been recently supported as an additional or possible alternative to the traditional methods of assessing the anaerobic threshold.[4,9–12] Simoes et al[9] have shown that blood glucose concentration exhibits a low point, a minimum, or nadir followed by an elevation during exercise of increasing intensity. They also suggested that this nadir in glucose is associated with similar physiological factors to those that underlie the lactate and ventilatory thresholds. This nadir in blood glucose during graded exercise has been coined the individual glucose threshold (GT)[4,9,10] and has recently been shown to be sensitive to changes as a result of physical training in some[12] but not all research.[13]

To date, the hormonal mechanisms underlying the GT have not been fully elucidated.[10,12] It has been suggested the minimum glucose and subsequent rise in blood glucose concentration may be due to glucoregulatory and counter-regulatory hormone responses associated with changing metabolic

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demands during exercise of increasing intensity.[10,14,15] Therefore, the purpose of this study was to investigate the response of blood glucose to graded cycling exercise combined with measurements of circulating insulin, glucagon, and cortisol concentrations. It was hypothesized that the glucose nadir would coincide with an elevated concentration of insulin, decreased concentration of glucagon, and an increased concentration of cortisol during graded exercise to exhaustion. A secondary purpose of the study was to compare the GT to other classical measures of lactate threshold (LT) and VT.

Methods

Participants

Twenty-two healthy, active males volunteered to participate in this investigation from a university and urban population. Participants were required to complete a Physical Activity Readiness Questionnaire and an informed consent form. The mean (±standard deviation, SD) age, height, and body mass of the participants was 24.5 (±4.0) years, 182.0 (±9.1) cm, and 84.8 (±17.4) kg, respectively. The participants also reported that they were all regularly involved in physical activity, participating in a mean (±SD) of 5.4 (±2.4) intense activity sessions per week. This investigation was reviewed and approved by a university research ethics board.

Experimental design

Each participant was provided with an orientation to the laboratory, allowed to try the exercise equipment, and requested to complete the required forms. During the second visit, height and body mass were measured using a tape measure and precision scale, respectively. Each participant then performed a standardized graded cycling exercise test to volitional exhaustion to measure peak oxygen consumption (VO_2 peak). Two to 5 days later, all participants completed a second standardized graded exercise test to assess their ventilatory, lactate, glucose, and hormonal responses. To establish a stable nutritional, glucose, and hydration baseline state, each participant was provided with one can (237 mL) of liquid Ensure (Abbott Laboratories, Abbott Park, IL, USA), which contains a standardized amount of carbohydrate (61%), fat (24%), and protein (15%) as well as vitamins and minerals as a pretest meal, 3 hours prior to this test. Each individual was also requested to consume 500 mL of water in small amounts (125 mL every 30 minutes) 2 hours prior to the test. Finally, each participant was required to refrain from any formal exercise 24 hours prior to each exercise test.

Exercise protocols

Peak VO_2 was assessed using a graded, incremental exercise to volitional exhaustion on a cycle ergometer (Monark Model 818E, Uppsala, Sweden) that required each participant to pedal at 75 rpm at an initial power output (PO) of ~74 watts followed by an increase of 37 watts every 2 minutes to volitional exhaustion. For this test, each participant was fitted with headgear to hold a Rudolph valve mouthpiece (Hans Rudolph, Shawnee, KS, USA) to collect expired air, which was analyzed in a metabolic measurement system (ParvoMedics True Max 2400, ParvoMedics, UT, USA). Gas concentration prior to and after each test was calibrated, whereas air volume was calibrated prior to each testing session. Volitional exhaustion was defined as the point at which the participant could not continue to exercise at a pedal rate above 50 rpm or stopped pedaling altogether due to fatigue, despite further verbal motivation. Peak VO_2 was defined as the highest VO_2 that was recorded during the exercise test and was associated with a respiratory exchange ratio greater than 1.15, achievement of age-predicted, or known maximum heart rate and volitional exhaustion in all cases. A standardized 5-minute cycling warm-up and cool-down at 37 watts was required. Heart rate was recorded every minute using a Polar Pacer heart rate monitor (Polar Electro, Kempele, Finland).

The experimental exercise test was graded using an incremental protocol to measure blood glucose, lactate, insulin, glucagon, and cortisol, as well as to subsequently identify the GT, LT, and VT. The pedal rate and incremental POs were the same as for the peak VO_2, test except that each exercise stage was 3 minutes in duration to allow for blood sampling. The collection of expired air with the same metabolic system and heart rate measurement were the same as previously described.

Threshold determination

GT, LT, and VT were determined during the experimental exercise test for each participant. Oxygen uptake and PO were used as the common dependent variables between the different threshold determinations. Two researchers independently and blind to the participants determined the threshold point in a random order based on the following guidelines. If the independent determinations of the PO at the threshold differed, a third researcher adjudicated the difference and confirmed the threshold.

The GT was defined as the lowest point or nadir in blood glucose concentration immediately preceding an increase during graded exercise, and the PO and VO_2 corresponding to this point was recorded.[9,10] Glucose concentration was plotted versus PO for this determination and visually determined as described previously. LT was defined as the PO and VO_2 above which a net increase in blood lactate production was observed according to the methodology of Wasserman et al.[16] The blood lactate response versus PO was graphed and the LT was determined as the first rise from the baseline blood lactate concentration. The PO and VO_2 at VT was determined using the V-slope method[16,17] from the graph of VCO_2 versus VO_2 provided by the metabolic measurement system software. Fig. 1 shows the ventilatory, glucose, and lactate responses versus PO and illustrates the individual threshold determination for one participant.

Blood collection and analysis

Prior to the experimental exercise test, a 22 gauge indwelling venous catheter (BD Medical, Franklin Lakes, NJ, USA) was placed in a forearm vein of each participant by a

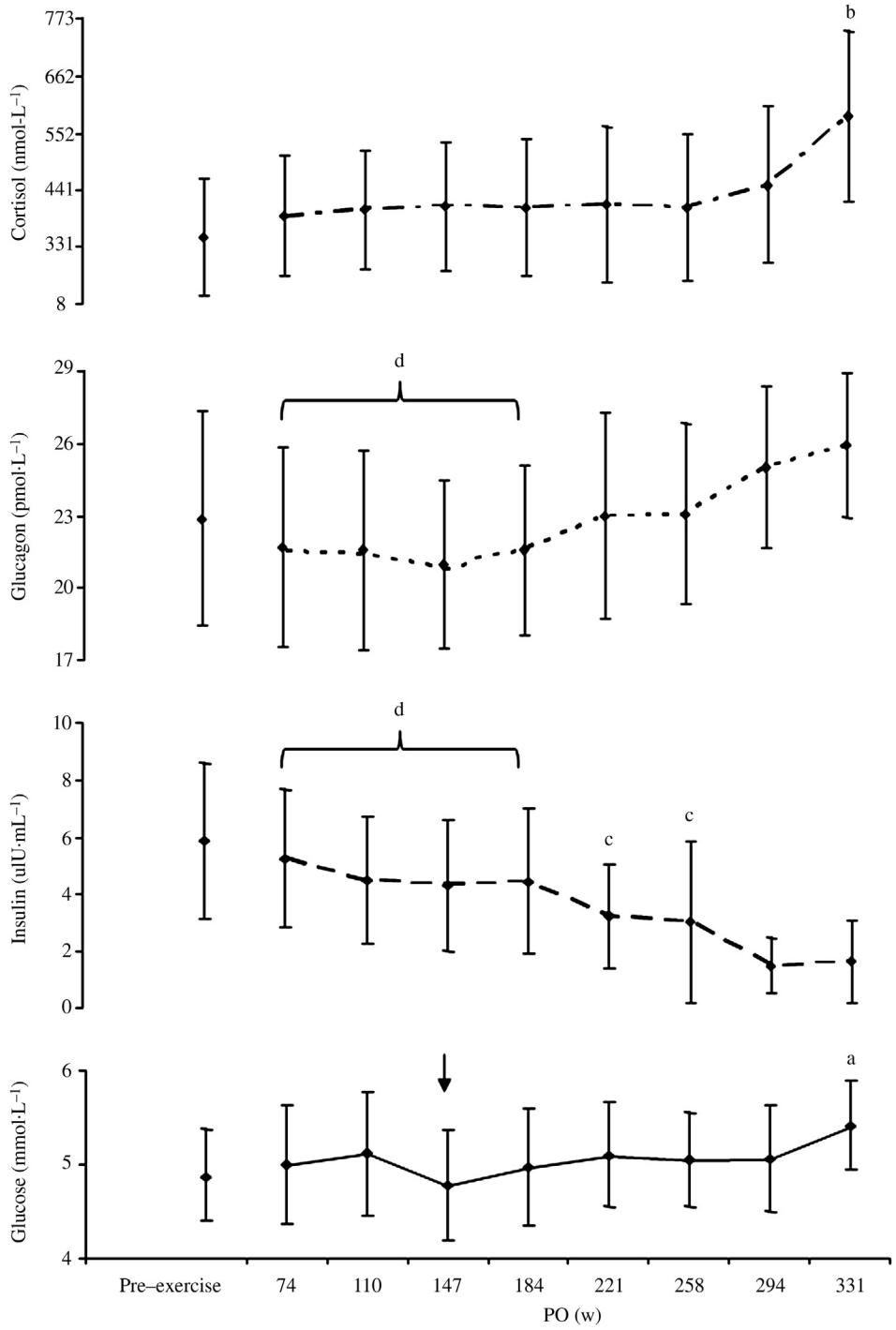

Fig. 1. Blood glucose, insulin, glucagon, and cortisol concentrations prior to and during graded exercise to exhaustion. Values are means ± standard deviation. Note: the arrow indicates the nadir in blood glucose. [a] Significantly different from rest, $p < 0.05$; [b] significantly different from rest and all other power outputs, $p < 0.05$; [c] significantly different from 74 watts, $p < 0.05$; [d] significantly different from the power output at 294 watts and 331 watts, $p < 0.05$. PO = power output; w = watt.

registered nurse. A resting blood sample was drawn, after which sterile saline (0.5 mL of 0.9% NaCl) was used to keep the line patent. In the final minute of each stage of the graded exercise test, 3 mL of blood was drawn to remove any saline in the line, followed by another 3 mL blood sample that was used for analysis. This was immediately followed by injection of another 0.5 mL of sterile saline to prevent any further clotting.

Immediately after each blood sample was taken, 0.25 mL of whole blood was added to 1 mL of ice-cold 8% perchloric acid, vortexed, and centrifuged at 3000*g* for 10 minutes prior to being frozen for later analysis of lactate concentration. Two 50 μL micro-hematocrit tubes were filled from each blood sample, centrifuged for 5 minutes in a microcentrifuge and hematocrit was determined as the ratio of packed red blood

cells to total plasma volume and expressed as a percentage. The remaining whole blood was allowed to clot at room temperature (~30 minutes) and then centrifuged at 3000*g* for 10 minutes. The serum was frozen and stored at −80°C until analysis. All blood samples were analyzed in duplicate within the same assay. Serum glucose and lactate concentration were assayed enzymatically using a spectrophotometer, according to the procedures outlined in Bergmeyer.[18] Serum cortisol, insulin, and glucagon were determined using commercially available radio-immunoassay kits (Diagnostics Products Corporation, Los Angeles, CA, USA). The mean coefficient of variance for the glucose, glucagon, insulin, cortisol, and lactate assays was 2.4%, 6.7%, 8.2%, 5.0%, and 2.4%, respectively.

Statistical analysis

The blood GT was identified in 20 of the 22 participants, whereas LT and VT were identified in all participants. Thus, any statistical comparisons based on dependent variables associated with the glucose nadir were conducted on a sample of 20 participants, whereas all other data analyses were carried out with a sample size of 22 participants. Statistical analyses were performed using a commercially available statistical software package (STATISTICA, Oklahoma City, OK, USA). Two separate one-way analysis of variance tests with repeated measures were used to compare the oxygen consumption and power output of the three different threshold determinations. Separate one-way analyses of variance with repeated measures were conducted to determine the differences in concentrations across common measurement times (at rest and each power output during the graded threshold exercise test). Significant F ratios were further examined with a Newman Kuels multiple comparison procedure and alpha was set *a priori* at $p < 0.05$ for all analyses.

Results

The blood glucose, insulin, glucagon, and cortisol concentrations are illustrated in Fig. 1. The lowest point in blood glucose and glucagon concentration occurred at the same PO during the graded exercise test and this occurred at the same time at which the insulin and cortisol were unchanged. Blood glucose was significantly higher at the final PO in comparison to rest. Insulin concentration was significantly lower after 221 watts compared to the first four power outputs. Glucagon concentration at the first four power outputs during the graded exercise test were lower than the final two power outputs ($p < 0.05$). Cortisol remained unchanged during the graded exercise test but was significantly elevated at the final PO in comparison to at rest or all other exercise power outputs.

The mean absolute and relative VO_{2peak} were 4.33 (±0.56) $L \times min^{-1}$ and 52.7 (±9.4) $mL \times kg^{-1} \times min^{-1}$, respectively, for all participants. The mean PO and VO_2 at VT were 210 (±33) watts and 2.90 (±0.49) $L \times min^{-1}$; at GT were 164 (±31) watts and 2.24 (±0.39) $L \times min^{-1}$; and at LT were 178 (±33) watts and 2.49 (±0.46) $L \times min^{-1}$, respectively (see Fig. 2). The PO and

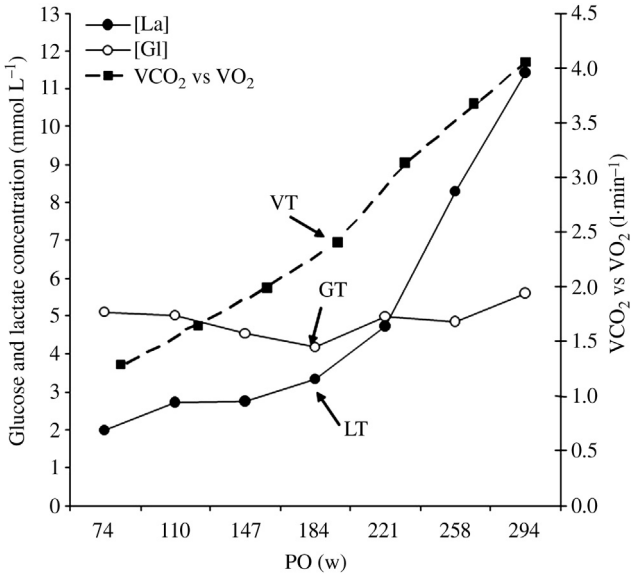

Fig. 2. The individual glucose, lactate, and ventilatory thresholds for a single participant. The glucose and lactate thresholds occurred at the same time and prior to the ventilatory threshold. GT = glucose threshold; LT = lactate threshold; PO = power output; VCO_2 = volume of carbon dioxide produced; VO_2 = volume of oxygen consumed; VT = ventilatory thresholds; w = watt.

VO_2 at GT and LT were not significantly different from each other but were both one PO lower than VT ($p < 0.05$).

Discussion

The primary purpose of the present study was to examine the underlying glucoregulatory hormone responses associated with the nadir in blood glucose or GT. It was found that the nadir in blood glucose during exercise occurred at the same PO as the nadir in glucagon, but prior to the PO at which insulin concentration began to significantly decrease during graded exercise, adding support to our hypothesis. Furthermore, our findings confirmed that the nadir in serum glucose during graded exercise occurred at a similar power output and oxygen uptake to the LT[9,12] but that both the GT and LT occurred immediately prior to the intensity of exercise that elicited VT.

The homeostatic regulation of blood glucose is primarily influenced by regulatory and counter-regulatory hormone concentrations, such as insulin, glucagon, and cortisol, as well as catecholamines (epinephrine and norepinephrine). The response of these hormones to exercise of sufficient duration and increasing intensity are well-documented.[19–27] Rocha et al[12] suggested that the response of blood glucose leading to its nadir during exercise of increasing intensity was likely to be associated with an enhanced translocation of GLUT-4 proteins in skeletal muscle stimulated by intracellular mechanisms as well as insulin.[28] It has been further suggested that following the glucose nadir, there is a glucagon-stimulated increase in liver glucose output, a subsequent inhibition of insulin release and greater reliance on muscle glycogen stores to support the increased rate of glycolysis during exercise of increasing intensity.[9,10,29,30] These responses are supported by

the present study, because the nadir in serum glucose concentration paralleled a concomitant increase in serum glucagon combined with a subsequent decrease in serum insulin concentration. The nadir in glucagon at the same time that insulin levels were maintained would suggest that hepatic glucose production was unable to counter the greater glucose uptake by working skeletal muscle.[9] Subsequent to the GT, the increased production of glucagon corresponding with declining insulin levels would suggest that hepatic glucose production was increased to establish euglycemia as exercise intensity increased. Finally, it has been shown that circulating cortisol concentration remains stable during low- and moderate-intensity exercise of short duration and increases at higher intensities of exercise of sufficient duration.[7,20,31,32] The present study supports this latter research, as cortisol did not show any significant changes until the highest exercise intensity during the test that also corresponded with the highest level of plasma glucose. This suggests that the gluconeogenic role of cortisol in increasing blood glucose levels is likely to be more important at higher intensities of exercise.

We recognize that circulating levels of hormones may reflect changes in production, binding, and removal. The data in the present study do not allow us to determine the interaction of these factors and the influence this would have on our findings. Also, it has been shown that catecholamines are known to stimulate glycogenolysis and to increase during graded exercise.[26,33] Thus, the role that the catecholamines may have had on the blood glucose responses in the present study is not known. Furthermore, it is important to point out that the blood GT was not identified in two individuals, even though LT and VT were identified in all participants. This may suggest that the identification of a GT is not always possible.[13]

Our finding of a nadir in serum glucose during graded exercise was in agreement with other studies that have observed a similar phenomenon despite using different exercise protocols.[9–12] Similar to the findings of Simoes et al,[9] no differences were observed between power output and oxygen uptake at the GT and LT, indicating that the nadir in serum glucose was similar to the power output at the time of the marked increase in blood lactate concentration, indicating an increased rate of glycolysis within skeletal muscle. Furthermore, Simoes et al[10] determined that VT and GTs also occurred at similar exercise intensity. This was in contrast to the present results, because the GT and LTs occurred at a power output immediately prior to that which elicited the VT. However, research has shown that indicators of the VT can occur at a higher intensity of exercise compared to the LT and was likely due to the increased production of CO_2 because of bicarbonate buffering of hydrogen ions as the rate of glycolysis increases.[2,16,17]

In conclusion, the results of this study demonstrated that the nadir in glucose occurred at the same time that glucagon concentration was at its lowest concentration, and both these responses occurred prior to a decrease in circulating insulin concentration during exercise of increasing intensity. The increase in blood glucose subsequent to the nadir was at least partly due to an increase in hepatic glucose output as evidenced by the increases in glucagon and cortisol concentration, but also due to a decrease in insulin. In addition, our findings indicate that the nadir in serum glucose during graded cycling exercise occurred in a predictable fashion compared to other accepted indicators of anaerobic threshold (e.g., LT and VT). This research provides further understanding of glucose control during graded exercise to exhaustion and supports other research[9,10] indicating that GT may be used as an additional marker of the individual LT and VT.

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